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FROM

THE INSTITUTE FOR MEDICAL RESEARCH,

FEDERATED MALAY STATES.

No. 13.

THE BACTERIOLOGY

OF

DYSENTERY IN MALAYA

BY

HENRY FRASER, M.D. ABERD.

Director, Institute for Medical Research.

Singapore

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CONTENTS.

THE BACTERIOLOGY OF DYSENTERY IN MALAYA.

IN this country dysentery is responsible for a large amount of the sickness and mortality. In 1914 there were treated in the Government hospitals 5,982 cases and 1,429 died. During the same period 5,355 deaths were recorded under the head of dysentery and diarrhœa, being 13.42 per cent. of the total recorded deaths. The hospital returns do not distinguish between amœbic and non-amœbic dysenteries, but in some hospitals systematic examinations of the stools are made and in a considerable number of cases amœbæ are found. In the District Hospital, Kuala Lumpur, the examinations are made by Mr. R. W. Lazaroo, an experienced and careful observer. He found in 1914 that in 132 out of 459 cases amœbæ were present and in 1915 amoebae were found in 117 out of 360 cases. Investigations were undertaken to determine the causative organisms in the non-amœbic cases.

At the outset of an inquiry on bacillary dysentery difficulty is encountered in the determination of the characters of several of the organisms which have been incriminated. In 1898 Shiga isolated the bacillus, with which his name is associated, from the stools of patients suffering from dysentery; the bacillus was agglutinated by the patient's serum. Following the work of this investigator, Flexner in 1900 announced the isolation of a bacillus from cases of dysentery in Manila and stated that the bacillus agreed in its morphological, cultural and pathogenic properties with the bacillus isolated by Shiga from the epidemic dysentery prevailing in Japan.

Shiga believed his bacillus to be motile and Flexner ascribed moderate motility to his bacillus. Flexner recorded the production

of acid in glucose-media and that lactose and saccharose were not fermented gaseously. Strong in 1900 recorded, as the result of work in Manila, the isolation of specific bacilli from cases of dysentery and Kruse in the same year published an account of his researches.

Discussions and investigations ensued on the identity or otherwise of these various organisms. By means of agglutination-reactions it was shown that the bacilli of Shiga and Kruse were identical and that the Flexner and Strong bacilli belonged to two other groups. It was observed that the organisms of Shiga and Kruse did not ferment mannite and that the organisms of Flexner and Strong did ferment it.

In 1903 Hiss and Russell described their Bacillus "Y" which fermented mannite and maltose. From this time the confusion of the mannite-fermenting organisms dates, the custom being established of comparing the reactions on sugar-media of freshly isolated strains with those of strains which had been isolated years previously. It was not recognized that the powers of the recently isolated organism to attack sugars could differ from those exhibited by the organism after a prolonged saprophytic existence. The method had the apparent recommendation of simplicity, other sugars were added to the list and practically every investigator found it possible in this way to create new varieties of the dysentery bacillus.

Investigators have consistently sought for the causative organisms of dysentery and allied conditions among the non-lactose fermenters. As regards the etiological significance of these organisms the evidence is by no means complete, but the results of numerous investigations make their connexion with the disease probable.

The non-lactose fermenters, to which the power of producing dysentery has been ascribed, are non-motile, in size and shape comparable to the typhoid bacillus and do not liquefy gelatine.

They all ferment dextrose, levulose and galactose, Shiga's bacillus does not ferment mannite, the others do. The mannite-fermenters vary in their action on maltose, saccharose and dextrin. It is difficult from the literature to determine the reactions of the different types which have been described. They are apparently limited only by number of possible combinations of the sugars and the following varieties may exist:—

Type	Mannite.	Maltose.	Saccharose.	Dextrin.
1	−			
2	+	+		
3	+		+	
4	+			+
5	+	+	+	
6	+	+		+
7	+		+	+
8	+	−	+	+

Bacillus "Y" according to Hiss and Russell is represented by type 2, others have found that it fermented dextrin (type 6), others have found that it did not ferment maltose (type 1), while others have observed that it fermented saccharose (type 3). *Bacillus dysenteriæ*, Flexner, may give the reactions of types 4, 6 and 8. *Bacillus dysenteriæ*, Strong, may apparently give the reactions of types 3, 5, 7 and 8.

Preliminary Investigation.

It is stated that the isolation of dysentery bacilli from the stools is a simple matter, more especially in the early stages of the disease, when a smear from the stool may yield practically a pure culture of the bacillus. The organisms being non-lactose fermenters, it is only necessary to smear a small quantity of the discharge on a large Petri dish containing alkaline lactose-agar tinted with an indicator. After incubation the colonies are selected which have not fermented lactose, and tested on sugar-media. If the reactions agree with one or other of the types previously indicated confirmatory evidence can be obtained by the use of specific agglutinating sera.

The media of Conradi-Drigalski without crystal violet, of Endo, and of MacConkey have each their advocates. After a series of trials it was decided to employ that of Conradi-Drigalski without crystal violet. The medium was poured into large Petri dishes and allowed to dry by exposure to the air ; it was then smeared with a portion of the discharge by means of a platinum wire which had been flattened into spatula-form and bent at an angle of 135 . Bent glass-rods, which have been recommended, were not found to be more useful than the platinum spatula, which has, moreover, the advantage of being readily sterilized. The inoculated plates were incubated for twenty-four hours at 37°C. Agar-slopes were inoculated from the blue or reddish-violet colonies and sugar-media and litmus-milk were inoculated from the cultures. Difficulty was encountered in the determination of the time which must be allowed to elapse before fermentation was to be considered complete. In the case of the aldehydes, dextrose and galactose, the ketone levulose and the alcohol mannite, fermentation, as represented by the production of acid, was invariably evident after twenty-four hours. In the case of the disaccharides, maltose and saccharose,

and the polysaccharide, dextrin, the changes which must take place before acid can be formed are more complex. Theoretically they must be inverted into aldehydes and ketones which are then oxidised. One strain of an organism may be able to produce these changes more rapidly than another of the same kind, and it is an arbitrary method which classifies the bacilli in accordance with their action on these substances after intervals ranging from one to fifteen days or longer. Organisms were found which fermented maltose after some days; when retested they were able to do so in twenty-four hours.

From the 15th May to the 15th August, 1914, the stools from every case of non-amœbic dysentery admitted to the District Hospital, Kuala Lumpur, were examined; during the last month the amœbic cases were included. Nineteen cases of amœbic dysentery were investigated and from two of them dysentery bacilli were cultivated. One hundred and five non-amœbic cases were investigated; from sixty-one of them dysentery bacilli were not obtained. Four may have been cases of acute diarrhœa but fifty-seven of them were clinically cases of dysentery: some may have been amœbic cases, despite the fact that on repeated examinations amœbæ were not found.

From the remainder, forty-four cases, dysentery bacilli were cultivated. In six cases the *Bacillus dysenteriæ*, Shiga, was present. There were obtained from thirty-eight cases mannite-fermenting organisms which possessed the characters necessary for their inclusion among the dysentery bacilli. If the organisms which only fermented mannite are considered as one variety, the number of other varieties is seven, which is the number of possible combinations of maltose, saccharose and dextrin. The organisms which fermented maltose after 24 hours or after some days and did not ferment dextrin may be considered to correspond to the bacillus "Y" of Hiss and Russell, but in every instance when retested at intervals ranging from a month to a year after isolation they fermented maltose and dextrin after 24 hours and then

presumably corresponded to the *Bacillus dysenteriæ*, Flexner. The organisms had not been trained to ferment maltose and dextrin, they had been cultivated on ordinary nutrient agar. Strain 2740 fermented maltose after 48 hours, retested two months later, it fermented maltose and dextrin in 24 hours. Strain 3157 fermented maltose after 3 days, retested one month later, it fermented maltose and dextrin in 24 hours. Some strains, such as 3723, did not ferment maltose or dextrin and when retested a year later had not acquired the power to do so. Strain 4130 fermented saccharose after 48 hours, but a year later it had lost the power to do so. Two strains were isolated from Case 4056, one strain fermented saccharose after 24 hours, the other fermented maltose and dextrin after 24 hours and saccharose after 48 hours; both of these strains may be considered to correspond to the *Bacillus dysenteriæ*, Strong.

Strain 2964 fermented dextrin after 12 days; retested one month later it fermented dextrin in 24 hours; this might be regarded as a new variety of dysentery bacillus. Sorbite is stated not to be fermented by the bacilli of Flexner and Hiss and Russell. It was tried with 35 freshly isolated strains and in 7 cases sorbite was fermented after 24 hours. Classification on the basis of sorbite must therefore be rejected. Classification could only be accomplished by the creation of new varieties, but the inconstancy of the sugar-reactions did not justify this procedure. Indeed it was obvious, that by juggling with maltose, saccharose and dextrin and the times when fermentation was to be regarded as complete, new varieties could be created with ease. Theoretically it should be simpler, by means of these organisms, to convert the ketone radicle of a monosaccharide such as dextrose into carboxyl than to effect a similar conversion of the aldehyde radicle of a monosaccharide such as levulose or the oxidation of the hydroxyl radicle in an alcohol such as mannite. It should be simpler to effect the oxidation of a monosaccharide than of a disaccharide such as lactose or of a polysaccharide such as dextrin. But it is difficult to explain the results stated to be obtained with the alcohols sorbite, dulcite and adonite. It is an

attempt to reduce biological phenomena to the exactitude of chemical reactions and is fundamentally unsound.

Agglutinating serum was prepared from several strains of mannite-fermenting organisms ,but the only result from its use was that the organisms were then grouped together as one variety. It was decided, therefore, to resume the inquiry when agglutinating serum had been prepared from a single mannite-fermenting strain.

Preparation of Agglutinating Sera.

Strain 2843, a typical *Bacillus dysenteriæ*, Shiga, was selected for the preparation of one serum. Strain No. 2740 was selected for the preparation of the other serum. At the time of isolation this mannite-fermenting organism fermented maltose after 24 hours and after fifteen days had not fermented dextrin, it was presumably the bacillus "Y" of Hiss and Russell. Retested two months later, it fermented maltose and dextrin in 24 hours and was now presumably the *Bacillus dysenteriæ*, Flexner. Retested after one year the reactions had not changed.

Rabbits were employed for the preparation of the sera. The inoculations were made intravenously. Considerable difficulty was experienced in the preparation of the sera, more especially with Shiga's strain, until it was found that the first inoculations should be made with bacilli killed at 55°C and agglutinated or sensitized with specific sera, the task then became simple. The inoculations were made at intervals of four days and the quantities were gradually increased from small doses of killed agglutinated bacilli to killed bacilli and then live bacilli, until ultimately the growths from two or three 24-hour-cultures, suspended in salt solution, were injected at one time. Seven days after the last inoculation, the titre of the serum was determined and if satisfactory the animal was bled from the carotid into a large sterile test tube, the lower end of which had been drawn out to a nozzle at right angles with the tube.

In the subsequent inquiry the sera obtained from rabbit No. 6, which had been treated with strain No. 2843, and from rabbit No. 15, which had been treated with strain No. 2740, were employed. Serum No. 6 had a titre of 1—2,000 and tested with

two other strains of Shiga's bacillus gave the same titre. Serum No. 15 had a titre of 1—3,500 but when tested with six other strains giving exactly similar reactions on sugar-media the results were as follows:—

Strain.		Titre after 24 hours.
2740	...	... 1—3,500
3356	...	... 1—4,000
2781	...	... 1— 1,000
4137	...	... 1— 300
3439	...	... 1— 400
2810	...	... 1— 500
3552	..	... 1—4,000

The wide range was somewhat surprising but with a Hiss and Russell agglutinating serum obtained from the Lister Institute and stated to have a titre of 1—12,800, strain No. 2740 gave a titre of 1—500, and with a Flexner agglutinating serum, also obtained from the Lister Institute, and stated to have a titre of 1—3,000, strain No. 2740 showed an agglutination limit of 1— 500.

A Shiga agglutinating serum from the same source and titre unstated gave with strain No. 2843 an agglutination limit of 1—10 and with Shiga serum (B. W. & Co.) the titre was 1—500. With four other strains the results were as follows:—

Strain.	Shiga serum (Lister Inst.)	Shiga serum (B. W. & Co.) 1—2000 to 1—4000.
99	1—50	1—400
111	1—10	1—500
3334	1—10	1—200
3550	1—50	1—500

The serum from Messrs. Burroughs Wellcome was sent out by post and may have deteriorated in transit. The serum from the Lister Institute was sent out in cold storage.

Serum No. 6 gave an agglutination reaction of 1—50 with strain No. 2740 and Serum No. 15 gave an agglutination reaction of 1—10 with strain No. 2843. Tested with other intestinal organisms the sera gave no agglutination. A variety of dysentery organisms were tested with the sera of normal rabbits; in no case was agglutination observed beyond 1—10 and in the majority of cases there was no agglutination.

Method employed for the determination of agglutination reactions and of the titre of sera.

The macroscopical method was employed. Agar-slopes were smeared with the organism to be tested and incubated at 37° C for 24 hours. One cubic centimetre of sterile salt solution was added to a culture and the growth emulsified. After standing for half an hour the coarser particles had subsided and the supernatant emulsion was pipetted off. The serum in varying dilutions was mixed with an equal amount of the emulsion, the mixtures were drawn into capillary tubes according to Wright's method and left standing at room-temperature (29°C) for 24 hours, the limit of agglutination was then recorded as being the one in which agglutination was complete and the supernatant fluid clear. In cases of doubt the capillary tubes were inverted and after an hour the titre recorded.

Culture Media.

A further series of trials was made with the various media recommended for the isolation of dysentery bacilli.

The medium devised by MacConkey is a comparatively poor one for the growth of micro-organisms. Inoculated plates show, after incubation for 24 hours, colonies which are only pin-head in size and those of the dysentery bacilli are extremely minute and transparent. This medium not only retards the growth of the organisms but it also retards their activity on lactose and increases the difficulty in the isolation of the specific bacilli. Endo's medium and Conradi-Drigalski's medium without crystal violet are superior; on them the dysentery bacilli form much larger colonies which enable agglutination tests to be made with consequent saving of time and materials. Drops of diluted agglutinating serum (1—25) are placed on glass slides, the suspected colony is touched with a platinum-wire and the material removed is emulsified with the drop of diluted serum. Conradi-Drigalski's medium without crystal-violet is, in our experience, superior to Endo's medium. The modified Conradi-Drigalski medium is merely an alkaline lactose-litmus-agar containing nutrose and the omission of this substance does not affect the value of the medium, so that for the isolation of the dysentery bacillus from the stools it is sufficient to employ a 3 per cent. nutrient-agar standardized to + 10, and made alkaline by the addition of 2 c.c. of a 10 per cent. solution of anhydrous sodium carbonate to a litre of the medium. One per cent. of lactose is added. Azolitmin is preferable to litmus as an indicator. For the sugar reactions peptone-water tinted with azolitmin and containing the sugars in the proportion of one per cent. was used. Dextrose, levulose, galactose, mannite, maltose, lactose, saccharose and dextrin were employed. Levulose and galactose might well have been omitted. Litmus milk was also used but it furnished no additional information. The paradimethyl-amido-benzaldehyde test was used for the detection of indol. A 50 per cent. gelatine had to be employed for the determination of liquefaction, and motility was determined in 5 hour bouillon cultures.

Duration of the Investigation.

During the period extending from the 1st August, 1915, to the 31st December, 1915, every case of dysentery, amœbic and non-amœbic, admitted to the District Hospital, was examined bacteriologically. It was designed to make the examination as soon as possible after the admission of the patient; in some cases the clinical diagnosis would, therefore, be provisional and for this reason a few cases were included which, on a revision of the diagnosis, were considered not to be suffering from dysentery.

The stools of one hundred and twenty-three cases were investigated. Forty-four were cases of amœbic dysentery, that is, the stools were found to contain amœbæ. Sixty-seven were cases of non-amœbic dysentery. Twelve were considered not to be cases of dysentery. The cases as they occurred month by month are shown in tabular form.

Table 2.

Amœbic.	Non-amœbic.		Not dysentery.
	Dysentery bacillus isolated.	Dysentery bacillus not isolated.	
	AUGUST.		
2744		2729	
2823		2749	
2840		2849	
2898		2957	
2951		2914	
2981		3012	
	SEPTEMBER.		
3033	3057	3068	3093 Diarrhœa.
3042	3179	3114	3163 ,,
3073	2243	3159	3305 ,,
3120		3195	
3144		3271	
3152		3281	
3157		3307	
3205		3291	
3212			
3229			
2683			
3284			
3328			
3343			
3366			
3364			

TABLE 2—*Continued.*

Amœbic	Non-amœbic.		Not dysentery.
	OCTOBER.		
3418	3464	3423	3409 Diarrhœa.
3436	3497	3462	3461 Malaria.
3490	3500	3510	3615 Diarrhœa.
3637	3649	3625	
3627	3680	3610	
3583	3682	3640	
3761	3688	3648	
	3769	3729	
	3727	3730	
	3761	3767	
		3768	
		3775	
		3785	
	NOVEMBER.		
3836	3888	3792	3864 Malaria.
3859	1197	3796	4000 Diarrhœa.
3870	3521	3829	4031 Malaria.
3913	2171	4014	4063 Nephritis.
3924	4110	3988	4034 Pulmonary tuberculosis.
3942			
3960			
4016			
4056			
4085			
4101			
	DECEMBER.		
4194	2796	4160	4331 Diarrhœa.
4244	4245	4228	
4413	4304	4271	
4422	4368	4290	
	4372	4307	
	4432	4319	
	4315	4387	
	4469	4328	
		4460	

Amœbic cases.

From no case of amœbic dysentery in this series was a strain of the dysentery bacillus cultivated.

From nine of the cases fæces were received and from eighteen of the cases feculent mucus was received. From these materials it is difficult to isolate non-lactose fermenters. In seventeen cases the stools were either bloody or bile-stained mucus, or mucus, or purulent mucus. From these materials it is not, as a rule, difficult to obtain non-lactose fermenters but none of those isolated conformed to the characters of any of the recognised dysentery bacilli.

An attempt was made in some cases to determine the possibility of a mixed infection by testing the agglutinating power of the patient's serum for various strains of dysentery bacilli. The results are inconclusive. The possibility could not be excluded that the patient had suffered from bacillary dysentery on a previous occasion. Statements obtained from the patients on this point are not reliable. Equally unreliable are the statements as to the duration of the illness.

Serum from Case	Strain 3761	Strain 3709	Strain 3497	Strain 3464	Strain 2740	Strain 3957 (Shiga)	Strain 2843 (Shiga)	Duration of illness
3769	1-100	1—80	1—50	1-40				90 days
3836	1-20	1—10	1—10	1—10				15 days
3859	1-50	1—20	1—50	1—40	Nil			60 days
3870	1—20	1—20	1—20	1—50				Unknown
3913	1-10	1-20	1-20	1—20	1-10			90 days
3924	1-10	1-20	1—20	1-20	1—10			10 days
3942	Nil	Nil	1—50	1-20	Nil			10 days
4060	1-10	1-10	1—40	1-10	Nil			60 days
4194	1-20	1-20	1-20	1-40	1—20			3 days
4413	Nil	Nil	Nil	Nil	Nil	Nil	Nil	60 days
4422	Nil	Nil	Nil	Nil	Nil	Nil	Nil	15 days

Non-dysenteric Cases.

Twelve cases were considered to belong to this group. Seven were regarded as cases of diarrhœa, three were cases of malaria, one was a case of nephritis and one was a case of pulmonary tuberculosis. Dysentery bacilli were not obtained from these cases. In some the titre of the serum for various strains of mannite-fermenting dysentery bacilli was determined.

Serum from Case.	Strain 3761	Strain 3709	Strain 3497	Strain 3464	Strain 2740	
3864	1—20	1—10	1—20	1—20	Nil	Malaria
4000	1—20	Nil	Nil	1—20	Nil	Diarrhœa
4031	Nil	Nil	Nil	1—10		Malaria
4034	1—10	1—10	1—10	1—20	Nil	Pulmonary tuberculosis
4063	1—10	1—50	1—20	1—20	1—20	Nephritis

Previous attacks of dysentery cannot definitely be excluded. The titre of the serum varies with different strains of similar organisms and it is difficult to decide what is to be regarded as a positive reaction. In the course of the inquiry thirty-one strains of non-dysenteric bacilli were cultivated from twenty-two cases and tested with their homologous sera. In two cases (3775 and 3785) a positive reaction of 1—20 was recorded but in the others there was no trace of agglutination. From six of these twenty-two cases dysentery bacilli had been cultivated which gave reactions with their homologous sera ranging from 1—20 to 1—100. There may on occasion be difficulty in determining the exact titre of a serum but there is no difficulty in distinguishing between a positive and a negative reaction. It can therefore be contended, with the exception of case 4031, that these patients were at the time of investigation suffering from bacillary dysentery. Using five

strains of dysentery bacilli the results are perhaps more difficult to interpret than if only one strain had been employed, but they serve to show their variability. Rabbits inoculated with similar quantities of dysentery bacilli show extreme variations in the titre of their sera, and apart from the duration or severity of the disease there is no reason why these variations should not exist in the case of man. In view of the sharpness of the reactions an agglutination of 1—10 is quite as satisfactory as an agglutination of 1—100, and provided that the clinical condition accords with the laboratory result there is no reason why a reaction of 1—10 should not be regarded as positive.

Comparison of the agglutination-reactions of homologous sera with dysenteric and non-dysenteric organisms.

Serum of Case.	Dysenteric organisms.	Non-dysenteric organisms	Kind of Case.
1197	(1) 1—40	(1) nil, (2) nil.	Bacillary
2171	(1) 1—80, (2) 1—50, (3) 1—80, (4) 1—50.	(1) nil.	Do.
2790	(1) nil	(1) nil, (2) nil, (3) nil, (4) nil	Do.
2914	None isolated.	(1) nil.	Non-amœbic.
3025	Do.	Do.	Do.
3068	Do.	Do.	Do.
3430	Do.	Do.	Amœbic.
3462	Do.	Do.	Non-amœbic
3464	(1) 1—100	Do.	Bacillary.
3521	(1) 1—80, (2) 1—20, (3) 1—20, (4) 1—20	Do.	Do.
3767	None isolated	Do.	Do.

Serum of Case.	Dysenteric organisms.	Non-dysenteric organisms.	Kind of Case
3768	None isolated.	(1) nil.	Non-amœbic.
3775	Do.	(1) 1—20.	Bacillary.
3785	Do.	(1) 1—20.	Do.
3888	(1) 1—80, (2) 1—80, (3) 1—80.	(1) nil, (2) nil.	Do.
3913	None isolated.	(1) nil, (2) nil.	Amœbic.
3988	Do.	(1) nil.	Bacillary.
4014	Do.	(1) nil, (2) nil, (3) nil.	Do.
4016	Do.	(1) nil, (2) nil.	Amœbic.
4110	(1) 1—20.	(1) nil.	Bacillary.
4228	None isolated.	Do.	Non-amœbic.
4245	(1) nil, (2) nil.	Do.	Bacillary.

Non-amœbic cases.

Sixty-seven cases have to be considered. They may be grouped into (1) cases from which dysentery bacilli were not isolated (2) cases from which dysentery bacilli were isolated.

(1) Cases from which dysentery bacilli were not isolated.

There were forty-one cases from which dysentery bacilli were not obtained, table 2 indicates that these cases were more common in the early part of the inquiry and suggests that the failure to isolate the causative organism in some, perhaps the majority, of these was due to defects in technique. This is admitted, but in conjunction with the results of the preliminary investigation the failures show that the isolation of the dysentery bacillus is by no means the simple operation that some investigators would have us believe it to be. The clinician, in dealing with non-amœbic cases of dysentery, desires to know the causative organism and at one time it was hoped, with the aid of specific agglutinating sera, to furnish this information within 24 hours from the time of receiving the material, but this ideal has not been attained. Even when several large plates are used or when the examinations are repeated several times only a minute quantity of the evacuations can be examined and, in our experience, it is exceptional for the non-lactose-fermenters to exceed in numbers the lactose-fermenters. On the assumption that the dysentery bacilli are not normally intestinal saprophytes, the pathological condition of the mucosa, which in many cases has the consistence of a door-mat, suggests that these organisms might, on occasions, not be evacuated, or at least only in such relatively small numbers as to make their isolation difficult.

On a plate of litmus-lactose agar, smeared with material containing organisms of the mannite-fermenting group, it is exceptional to find that the colonies are blue; as a rule they are purplish

and on occasions red, which means that at the time of isolation they can ferment lactose in the medium which we employed. The non-recognition of this fact may account for some of the failures; its recognition accounts for some of the successes but it is obvious that the power to ferment lactose in this way introduces a very considerable element of chance into the work of isolation. If the colonies are tested with specific sera the element of chance may be minimised but it cannot be abolished. The number of colonies which develop on a plate spread with dysenteric material is, as a rule, very large; the opaque red colonies can be neglected, but the transparent red ones cannot all be tested in this way; equally so in a plate with many purplish colonies it is impossible to test them all.

It has been stated that the causative organisms are discharged in larger numbers at certain stages of the disease and, even admitting the unreliability of the history of the duration of the disease, the list shows that cases in every stage of the disease were examined. Some cases of amoebic dysentery may have been included but it is improbable that the majority were. Some, at least, of those cases in which a history of previous attacks was not obtained must have been suffering from bacillary dysentery as shown by the positive reactions obtained with the serum and various strains of dysentery bacilli.

Serum from Case	Strain 3761.	Strain 3709.	Strain 3497.	Strain 3464.	Strain 2740.	Strain 3057 (Shiga).	Strain 2843 (Shiga).	Duration of disease.
3767	1—80	1—20	1—40	1—20				20 days.
3775	1—200	1—200	1—200	1—160	1—80			60 days.
3785	1—200	1—50	1—100	1—50	1—40			6 days.
3792	1—10	Nil	1—50	Nil				15 days.
3988	1—50	1—50	1—50	1—40	1—40			60 days.
4014	1—50	1—40	1—80	1—50	1—20			6 days.

Serum from Case.	Strain 3761.	Strain 3709.	Strain 3497.	Strain 3464.	Strain 2740	Strain 3057 (Shiga).	Strain 2834 (Shiga).	Duration of disease.
4160	1—20	1—10	1—10	1—20	1—10			30 days.
4228	1—10	1—10	1—10	1—10	Nil	Nil	Nil	5 days.
4271	1—20	1—10	1—10	1—10	Nil	Nil	Nil	30 days.
4290	1—10	1—10	1—10	1—10	Nil	Nil	Nil	2 days.
4304	1—50	1—100	1—50	1—50	1—10	Nil	1—20	8 days.
4307	Nil	1—20	1—10	1—10	Nil	1—80	1—80	60 days
4319	1—20	1—20	1—10	1—20	1—10	Nil	Nil	90 days.
4328	1—20	1—20	1—20	1—10	1—10	1—20	1—40	6 days.
4331	1—10	Nil	Nil	Nil	Nil	Nil	Nil	75 days.
4387	Nil	1—20	1—20	1—20	1—40	1—10	1—20	6 days.

Even after months of practice dysentery bacilli could only be isolated from about half the cases of non-amœbic dysentery. It must therefore be concluded that in a considerable number of the cases either the specific colonies were overlooked or the bacilli were not present in the dejecta or were present in such small numbers as to make their isolation a matter of chance or were present as lactose-fermenters.

A very large number and variety of organisms were isolated and examined. Some of them from their sugar-reactions would have been classed as dysentery bacilli; they were rejected because of other differences such as motility, the power to liquefy gelatine, and the failure to react with specific sera. On the plate smeared with material from Case 3068 only blue colonies were seen: these were not agglutinated by serum 15 or serum 6. Four colonies were examined in detail, the organisms were non-motile, in size and shape comparable to the dysentery bacillus. The sugar reactions were those of the *Bacillus dysenteriæ*, Shiga, but indol was formed and the bacilli were not agglutinated by serum 6 or by the Shiga serum obtained from the Lister Institute.

On the plate obtained from Case 3271 there were some blue colonies; these were not agglutinated by serum 15 and serum 6. Three colonies were examined in detail. After 24 hours they gave the sugar-reactions of Shiga's bacillus, but after 4 days gas was produced in dextrose and levulose. Retested, gas was produced in dextrose in 24 hours.

On the plate prepared from Case 3648 there were several purplish colonies which were agglutinated by serum 15. Subcultures were made from three colonies and examined in detail. In size and shape the bacilli resembled the dysentery bacillus, the sugar-reactions were those of the mannite-fermenters, but they liquefied gelatine and were not agglutinated by specific sera. The patient died, and post-mortem, the conditions were those found in cases of bacillary dysentery. Unfortunately the homologous serum was not obtained but it is almost certain that subcultures were made from the wrong colonies.

From the first specimen from Case 3767 only lactose-fermenters were obtained. Similar results were obtained with second and third specimens. Among the colonies were some transparent red ones, subcultures were made from several of these, two produced indol and were unaffected by specific sera, but otherwise the characters were those of Shiga's bacillus. The homologous serum was tested with several strains of mannite-fermenters. The positive agglutination reactions indicated that the present attack was caused by organisms of this type; there was no history of previous attacks. It is possible that in this case the organism was a lactose-fermenter at the time of isolation and was overlooked.

On the plate prepared from Case 3610 only red colonies were seen, the colonies all looked alike, a subculture was made from one of them and tested. It had then, 48 hours after isolation, lost the power to ferment lactose, which shows the instability and unreliability of this character. The organism fermented mannite and saccharose, in litmus-milk acid and clot was produced after

six days, but it was motile and liquefied gelatine; it could not therefore be Strong's bacillus. On agar it gave a moist yellow growth unlike that of the dysentery bacillus. It was unaffected by specific sera. With the homologous serum it gave a reaction of 1—100 and the admission of this organism as a dysentery bacillus would be quite as reasonable as the admission of some other organisms.

The systematic employment of more than one large plate and of repeated examinations might have diminished the number of failures, but all of them could not have been changed to success. It is scarcely practicable to employ more than three plates for one specimen and it is not practicable to examine every stool passed by a case from which, after more than one examination, the bacillus has not been isolated. Prolonged practice and experience does lessen the number of failures but there is at present no method by which, with any degree of certainty, the dysentery bacillus can be isolated from the stools of a case of bacillary dysentery.

Number of Case.	Duration of Disease.	Character of material received.	Litmus-lactose Agar.	Dextrose.	Result.	Remarks.
2291	9 days	Feculent mucus	All lactose fermenters		Cured	
2729	7 days	Feculent mucus	All lactose fermenters		Cured	
2749	10 days	Blood-stained muco-pus	Some doubtful non-lactose fermenters	Acid and gas	Cured	
2849	2 months	Blood-stained muco-pus	Some non-lactose fermenters	Acid and gas	Cured	
2914	2½ months	Feculent mucus	Some doubtful non-lactose fermenters	Acid and motile	Cured	
2957	5 months	Feculent mucus	All lactose fermenters		Cured	
3012	5 days	Muco-pus	Some non-lactose fermenters	Acid and motile	Cured	
3025	3 days	Blood-streaked mucus	Some non-lactose fermenters	Acid and motile	Cured	

Num- of Case	Duration of Disease.	Character of material received.	Litmus-lactose Agar.	Dextrose.	Result.	Remarks.
3114	10 days	Feculent mucus	All lactose fermenters		Relieved	
3159	15 days	Muco-pus	Some non-lactose fermenters	Nil	Cured	
3195	1 month	Bile-stained mucus and blood	Some non-lactose fermenters	Acid and gas	Cured	
3271	10 days	Yellowish watery fluid with some flakes	Some non-lactose fermenters	Acid and gas after 4 days	Absconded	
3281	2 months	Bile-stained mucus	All lactose fermenters		Died	
3367	15 days	Mucus	All lactose fermenters		Cured	
3423	25 days	Feculent mucus	Some doubtful non-lactose fermenters	Acid and gas	Cured	
3462	9 days	Feculent mucus	All lactose fermenters		Cured	
3510	3 months	Muco-pus	All lactose fermenters		Died	
3610	1 month	Muco-pus	Some non-lactose fermenters	Acid and gas	Cured	
3640	3 days	Muco-pus	All lactose fermenters		Died	Stools twice examined
3729	4 months	Blood-streaked muco-pus	All lactose fermenters		Died	Stools twice examined
3730	4 months	Feculent mucus	All lactose fermenters		Died	
3768	6 days	Blood-streaked mucus	All lactose fermenters		Cured	
3775	2 months	Blood-streaked muco-pus	Some non-lactose fermenters	Acid and gas		
3785	6 days	Feculent mucus	Some non-lactose fermenters	Acid and gas	Died	

Num- of Case.	Duration of Disease.	Character of material received.	Litmus-lactose Agar.	Dextrose.	Result.	Remarks.
3702	15 days	Feculent mucus	All lactose fermenters		Absconded	Stools twice examined
3746	15 days	Mucus	Some non-lactose fermenters	Acid and gas	Died	
3821	3 days	Feculent mucus	All lactose fermenters		Absconded	Stools twice examined
3988	2 months	Feculent mucus	All lactose fermenters		Absconded	Stools twice examined
4014	6 days	Muco-pus	All lactose fermenters		Died	Stools twice examined
4100	1 month	Bile-stained mucus	All lactose fermenters		Cured	Two plates smeared
4228	5 days	Blood-streaked mucus	Some non-lactose fermenters	Acid and gas	Died	Stools twice examined. Three plates smeared
4271	1 month	Mucus	All lactose fermenters		Died	Two plates smeared
4290	2 days	Feculent mucus	All lactose fermenters		Cured	Two plates smeared
4307	2 months	Feculent mucus	All lactose fermenters			Stools twice examined. Four plates smeared
4319	3 months	Feculent mucus	Some non-lactose fermenters	Acid and gas	Died	Stools twice examined. Four plates smeared
4328	6 days	Blood-stained mucus	All lactose fermenters			Stools twice examined. Eight plates smeared
4387	6 days	Muco-pus	All lactose fermenters		Died	Stools twice examined. Four plates smeared
4390	7 days	Feculent mucus	All lactose fermenters		Cured	Four plates smeared

Number of Case.	Duration of disease.		With Flexner serum (Lister Inst.) (1—3000)	With Hiss & Russell serum (Lister Inst.) (1—12800)	With Shiga serum (Lister Inst.)	Result.
3068	10 days	M	Nil	Nil	Nil	Cured
3648	2 days	F	Nil	Nil		Died. Enteric colitis; granular type
3767	20 days	M	Nil	Nil	Nil	Cured

Number of Case.	Duration of disease	Character of material received.	Litmus-lactose Agar.	Dextrose	Mannite	Maltose	Saccharose	Dextrin	Indol	Gelatine	With homologous serum	With serum No 15 (1—3500)	With serum No. 6. (1—2000)	With Flexner serum (Lister Inst.) (1—3000)	With Hiss & Russell serum (Lister Inst.) (1—12800)	With Shiga serum (Lister Inst.)	Result
3068	10 days	Muco-pus	All non-lactose fermenters	Acid Non-motile	Nil	Nil	Nil	Nil	+	Nil		Nil	Nil	Nil	Nil	Nil	Cured
3648	2 days	Feculent mucus	Some non-lactose fermenters	Acid Non-motile	Acid	Acid	Nil	Nil	+	Liquefies		Nil		Nil	Nil		Died Enteric colitis; granular type
3767	20 days	Muco-pus	All lactose fermenters on three plates	Acid Non-motile	Nil	Nil	Nil	Nil	+	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Cured

(2) Cases from which Dysentery Bacilli were isolated.

There were 26 cases from which dysentery bacilli were isolated; this represents 40 per cent. of the non-amœbic cases. The percentage of successes was the same as in the preliminary investigation. The twenty-six comprised cases in every stage of the disease, just as in the preceding group, and the materials examined were similar in both groups. There is, therefore, no apparent reason for success in the one group nor for failure in the other.

From only two cases, Nos. 3057 and 4245, was the *Bacillus dysenteriæ*, Shiga, isolated. The total number of cases from which we have at any time isolated Shiga's bacillus is 10; in no case has the power to ferment lactose been observed at the time of isolation. With the specific serum (No. 6) prepared for Shiga's bacillus, the organisms gave a high titre, but with the Shiga's serum obtained from the Lister Institute the reactions were only 1—10 and 1—50. The latter serum was tested with five other strains of Shiga's bacillus with similar results. The titre of the serum was not stated, it was sent to this country in cold-storage and stored here in the refrigerator. Flexner and Hiss and Russell sera were received and stored in the same way; they were active and it is difficult to account for the results with the Shiga's serum. The results were better with the Shiga's serum obtained from Messrs. Burroughs Wellcome, but that serum, as previously noted, may have deteriorated in transit.

From the remaining 24 cases mannite-fermenting strains of dysentery bacilli were cultivated.

On the plates smeared with material from Cases 2790, 3080, 3727, 4315 and 4368 transparent red colonies were observed which were agglutinated by serum 15. The colonies contrasted sharply with the blue or purplish colonies and with the opaque red colonies of the organisms which are considered saprophytes. The redness

produced in the medium by the action of the latter colonies frequently extends to some distance in the adjacent medium and it was considered not impossible that the redness of the specific colonies was due to this extended action. If this was the explanation it would only show that the method of isolation was unsatisfactory. Subcultures on agar were prepared from the transparent red colonies and from these cultures lactose-peptone water was inoculated. In no case did the organisms then ferment lactose. When not red on the litmus-lactose agar the colonies are as a rule purplish; it is exceptional to find that they are blue; they have therefore in many cases some action on lactose and increased activity on this substance with consequent redness would not be surprising. The vagaries of the dysentery bacilli on sugars are not confined to lactose, thus strain 2243 fermented mannite when freshly isolated but lost and did not regain that power. Strains isolated from Cases 3709 and 3761 fermented saccharose when freshly isolated but later lost this power.

From a mixture made with stock cultures of lactose and non-lactose fermenters and spread on a plate of litmus-lactose-agar only sharply defined red and blue colonies are obtained, because the organisms are more constant in their action, but with a similar mixture of organisms from a dysenteric stool it is exceptional to find sharp differentiation.

Mannite, like dextrose, levulose and galactose, was invariably fermented after 24 hours, but the activities on maltose, saccharose and dextrin were subject to great variations. The strains isolated from Cases 3179 and 3727, fermented maltose after two to five days. They might, therefore, be considered to correspond to the bacillus of Hiss and Russell, but when retested three months later, they fermented dextrin in 24 hours and might then be considered to be of the Flexner type.

The strains isolated from Case 3464 did not ferment maltose after 5 days, but, retested two months later, maltose was fermented after 3 days. It might be contended that these were really

maltose-fermenters at the time of isolation and that an insufficient time had been allowed for the action of the organisms on that substance. In a moist tropical climate contaminations are a constant source of trouble, especially with liquid-media. The liquid evaporates and permeates the plugs, moulds form and drop into the liquid and observations made on liquid-media after some days are liable to be erroneous. No method has been found to overcome this difficulty. The limit of five days was employed, but even this limit has on occasions been productive of doubtful results and longer periods cannot be trusted.

The strains isolated from Cases 3521, 4110 and 4368 fermented neither maltose nor dextrin after 5 days. Retested two months later, they had not acquired the power to do so. These strains could not therefore be considered to correspond either to the bacillus "Y" of Hiss and Russell or to Flexner's bacillus.

One strain isolated from Case 3709 fermented saccharose after 24 hours and maltose after 3 days. It might, therefore, be considered to correspond to the Strong's bacillus but retested 14 days later, it had lost the power to ferment saccharose and fermented maltose and dextrin after 24 hours like a Flexner's bacillus.

One strain isolated from Case 3761 fermented saccharose after 24 hours. Retested 14 days later, it had lost the power to ferment saccharose and, like the other strains from this case, did not ferment maltose and dextrin. It had therefore lost the characters of Strong's bacillus.

One strain isolated from Case 3888 fermented saccharose after 2 days. Retested, it did not do so but, like the other strains from this case, fermented maltose after 24 hours and dextrin after several days.

On the plate prepared from the first specimen of Case 3949 only one purpish colony was seen and it was not agglutinated by

specific sera. On the plate from the second specimen there were only red colonies. On the plate from the third specimen there were three purplish colonies which were agglutinated by serum 15. Subcultures were made from these but two of them produced acid and gas in dextrose and were rejected. The third fermented saccharose and produced acid and clot in milk in 24 hours, after 2 days maltose and dextrin were fermented. With serum 15 this culture gave a titre of 1—500, with the Hiss and Russell serum (Lister Institute) it gave a titre of 1—3,000 and the same titre with the Flexner serum (Lister Institute). The culture was believed to be impure and on a plate of litmus-lactose agar it was found to be a mixture of lactose and non-lactose fermenters, cultures were made from the latter colonies, which preponderated. The sugar-reactions were of the Flexner type and with the agglutinating sera the results were the same as before, which shows that the presence of lactose-fermenters in moderate numbers does not affect the agglutinations. Similar observations were made in Cases 4315 and 4432. The lactose-fermenters did not affect the appearance of the cultures on agar, and as the rate of growth of both kinds of organisms is apparently the same, they can continue in the mixture in the same proportion. The results suggested that the single strain of *Bacillus dysenteriæ*, Strong, which is in existence, might be a mixture of this type. A culture of it was kindly supplied by the Director, The Bureau of Science, Manila. The culture was plated and found only to consist of non-lactose-fermenting organisms; it fermented mannite but did not ferment either maltose, saccharose or dextrin, and did not produce acid and clot in milk; indol was produced. Tested with specific agglutinating sera the results were as follows:—

With serum No. 15.	With Flexner serum (Lister Inst.)	With Hiss and Russell serum (Lister Inst.)
1—100	1—1,500	1—4,000

The *Bacillus dysenteriæ*, Strong, is therefore not a distinct variety or type of the mannite-fermenting bacilli.

Serum 15 was prepared from an organism which, at the time of isolation, fermented maltose like a Hiss and Russell organism, but later fermented maltose and dextrin like a Flexner's bacillus. The organisms isolated of this type gave the following results with this serum and with the sera obtained from the Lister Institute and Messrs. Burroughs Wellcome.

	With serum 15 (1—3,500.)	With Flexner serum (Lister Inst.) (1—3,000.)	With Flexner serum (B. W. & Co.) (1—2,000 to 1—4,000.)	With Hiss & Russell serum (Lister Inst.) (1—12,800.)
2790	1— 200	1— 200	1— 100	1—1,500
3179	1— 500	1— 500	1— 50	1—3,000
3680	1— 200	1—1,000	1—100	1—4,000
3688(1)	1— 10	1— 500	1—300	1—2,000
3709(2)	1—2,000	1— 500	1—100	1— 500
3727	1— 500	1—1,500	1—400	1—1,500

The strains, which fermented maltose and dextrin at the time of isolation, gave the following results.

1197	1—2,000	1—1,500	1—100	1—1,500
2243	1— 300	1— 400	1— 10	1— 400
3049	1— 500	1—3,000	1—300	1—4,000
3497	1— 200	1— 100	1—500	1—3,000
3500	1— 200	1— 400	1—200	1—2,000
3680	1— 200	1—1,000	1—100	1—4,000
3682	1— 200	1— 500	1—300	1—1,500
3688(2)	1— 50	1— 500	1—300	1—2,000
3888(1)	1—2,000	1—1,000	1—200	1—1,000
3888(5)	1—2,000	1—1,000	1—200	1—1,500
4304	Nil	1— 10	1— 10	1— 500
4315	1— 200	1— 500	1—200	1—2,000
4372	1— 200	1— 200	1—200	1—4,000
4432	1—1,500	1— 500	1—200	1—1,000
4469	1—2,000	1— 500	1—300	1— 50

The strains, which did not ferment maltose and dextrin at the time of isolation and did not acquire the power to do so, gave the following results.

3521	1—300	1—400	1—100	1—3,000
4110	1—200	1—300	1—500	1— 500
4368	1—200	1—400	1—500	1—4,000

A strain, which did not ferment maltose at time of isolation but later acquired power to do so, reacted thus.

3464	1—200	1—400	1—200	1—1,500

A strain, which did not ferment maltose at time of isolation but later acquired power to ferment maltose and dextrin, reacted thus.

3688 (4)	1—10	1—500	1—300	1—2,000

A strain, which fermented maltose and saccharose at time of isolation, later lost the power to ferment saccharose and acquired power to ferment dextrin, reacted thus.

3709 (1)	1—2,000	1—400	1—100	1—500

A strain, which fermented maltose at time of isolation but later lost the power to do so, reacted thus.

3761 (2)	1—300	1—700	1—400	1—6,000

A strain, which fermented saccharose at time of isolation but later lost the power to do so, reacted thus.

3761 (1)	1—300	1—700	1—400	1—6,000

A strain, which fermented maltose, saccharose and dextrin at time of isolation but later lost the power to ferment saccharose, reacted thus.

3888 (2)	1—3,000	1—1,500	1—200	1—1,000

A strain, which fermented dextrin at time of isolation reacted thus.

2171 (1)	1—300	1—300	1—100	1—1,500

The titre of serum No. 15 may have fallen, only one strain 3888 (2) gave a reaction of 1—3,000 with it. The organisms, which theoretically ought to have given high titres with the serum, gave titres ranging from 1—10 to 1—2,000. They all possess the same biological characters and 3688 (1) cannot be excluded on account of a reaction of 1—10.

In the next group there is an equally wide range, strains 3688 (2) and 4304 have the same biological characters as the others and cannot be excluded. It might be contended that these strains really belonged to the Flexner type but the reactions with a Flexner serum show equally wide variations. Compared with the reactions with Hiss and Russell serum the range is equally wide.

Strains 3521 and 4368 cannot be considered as Hiss and Russell types and yet the serum-reactions entitled them to be considered as such. Strain 4110 cannot be excluded on account of the low titre. The reactions of these strains with the Flexner serum when compared with the reactions of typical Flexner bacilli would equally justify their consideration as Flexner types.

Strain 3464 might be considered a typical Hiss and Russell, but with that serum it only gave a titre of 1—1,500 and contrasts markedly with strain 4368 which gave a titre of 1—4,000 with the same serum and could not be considered a Hiss and Russell strain.

Strain 3761 (2) might be considered to have been a Hiss and Russell one at the time of isolation, although later it lost the power to ferment maltose. With the Hiss and Russell serum it gave a titre of 1—6,000, but 3761 (1) from the same case gave the same titre and this strain did not acquire the power to ferment maltose. It had at first the power to ferment saccharose but later lost it.

The reactions obtained with all strains and serum prepared from a single strain of a mannite-fermenter show such extreme variations as to justify the conclusion, already arrived at from the

sugar-reactions, that the grouping of the mannite-fermenters into types or varieties according to their actions on maltose, saccharose and dextrin is unsound. The conclusion is emphasised by the results with the Flexner serum and Hiss and Russell obtained from the Lister Institute. They also show how unreliable the results must be of the worker who collects cultures isolated by other workers, studies and groups them by their sugar and serum-reactions.

The results obtained with the mannite-fermenters and the Shiga serum from the Lister Institute are curious; in one case it reacted up to 1—500 and in five cases it reacted up to 1—300. It is not as specific serum like that obtained from Messrs. Burroughs Wellcome.

The separation of the mannite-fermenters from the non-mannite-fermenters is much sharper with the sera prepared here from single strains. The objection to them is that on occasions they fail to react, thus a mannite-fermenter from Case 4304 gave no reaction with serum 15; it gave a reaction with Hiss and Russell serum from the Lister Institute, but unfortunately it was not an organism of that type.

The agglutinating power of the patient's serum was tested in a number of cases. The results show the variability of this reaction even in cases in which the disease has been present for approximately similar periods.

Serum from Case	Strain 3761	Strain 3709	Strain 3497	Strain 3464	Strain 2740	Strain 3057 (Shiga)	Strain 2843 (Shiga)	Duration of illness
1197	1—50	1—20	1—40	1—20	1—20			4 days
2171	1—20	1—50	1—40	1—20	1—10			7 days
2790	1—10	Nil	1—20	1—20	1—10	Nil	Nil	3 days
3521	1—20	1—10	1—20	1—20	Nil			2 days

Serum from Case	Strain 3761	Strain 3709	Strain 3497	Strain 3464	Strain 2740	Strain 3057 (Shiga)	Strain 2843 (Shiga)	Strain 2843 illness
3709	1—200	1—50	1—40	1—190	1—50			6 days
3888	1—50	1—20	1—40	1—50	1—20			5 days
4110	1—20	1—10	1 10	1— 10	Nil			2 days
4245	1—10	1—10	1—20	1—20	Nil	Nil	1—20	4 days
4315	1—20	1—10	1—20	1—20	1—20	Nil	1—20	15 days
4368	1—20	1—20	1—20	1—20	Nil	Nil	Nil	10 days
4372	Nil	Nil	Nil	Nil	Nil	Nil	Nil	15 days
4432	1—20	1—10	1—20	1—10	1—10	Nil	Nil	8 days
4469	1—10	1—10	1—10	1—20	1—20	1—10	1—10	1 year

The mannite-fermenting strains isolated from Case 2243 are peculiar in that they lost the power to ferment mannite. Shiga has investigated similar strains and suggested the creation of a new type but the agglutination reactions do not warrant this nor do they justify the consideration of these strains as intermediate to the non-mannite and the mannite-fermenters.

The term pseudo-dysentery has been applied to the disease caused by the mannite-fermenters on the grounds that the clinical conditions are less severe than those caused by Shiga's bacillus. Some cases ran a mild course, but others were severe and ended fatally. In no case could the disease be diagnosed as other than dysentery. The term pseudo-dysentery is misleading and should not be employed.

Number of Case.	Duration of disease.	Character of material received.	With Shiga serum (Lister Inst.)	With Shiga serum (B. W. & Co.) 1—2000 to 1—4000	With homologous serum.	Result.	Remarks.
3057	2 days	Blood and muco-pus	—10	1—100	1—10	Cured	
4245	4 days	Blood and mucus	—50	1—200	Nil	Discharged at patient's request	
1197	4 days	Feculent mucus	—300	Nil	1—40	Cured	
2171	10 days	Feculent mucus	—100	Nil	1—80	Died	
2243	2 days	Blood-streaked muco-pus	—10	Nil	1—20	Cured	Curious result with mannite. Retested it had not regained the power to ferment mannite
2790	3 days	Blood and mucus	—100	Nil	Nil	Died	Retested, fermented maltose and dextrin
3049	5 days	Feculent mucus	—500	Nil	..	Died	
3179	3 days	Bile-stained muco-pus	—50	Nil	1—80	Cured	Retested, fermented dextrin in 24 hours and maltose after 2 days

Number of Case	Duration of disease	Character of material received.	Litmus-lactose Agar.	Dextrose	Mannite	Maltose	Saccharose	Dextrin	Indol	With serum No. 15 1—3500	With serum No 6 1—2000	With Flexner serum (Lister Inst.) 1—3000	With Flexner serum (H. W. & Co.) 1—2000 to 1—4000	With Hiss & Russell serum (Lister Inst.) 1—12800	With Shiga serum (Lister Inst.)	With Shiga serum (H. W. & Co.) 1—2000 to 1—4000	With homologous serum.	Result.	Remarks.
3057	2 days	Blood and muco-pus	A few small blue colonies. Many red colonies	Acid	Nil	Nil	Nil	Nil	–	—10	1—2000	1—100	Nil	1—50	1—10	1—100	1—10	Cured	
2445	4 days	Blood and mucus	Practically all blue colonies	Acid	Nil	Nil	Nil	Nil	–	1—10	1—1000	1—100	Nil	1—50	1—50	1—200	Nil	Discharged at patient's request	
2197	4 days	Feculent mucus	One purplish colony. Numerous red colonies	Acid	Acid	Acid after 2 days	Nil	Acid	+	1—2000	1—10	1—1500	1—100	1—1500	1—100	Nil	1—40	Cured	
2171	10 days	Feculent mucus	Majority are medium blue colonies	Acid	Acid	Nil	Nil	Acid after 3 days	+	1—300	1—10	1—300	1—100	1 1500	1—100	Nil	1—80	Died	
2243	2 days	Blood-streaked muco-pus	All blue colonies	Acid	Acid but alkaline after 48 hours	Acid	Nil	Acid	+	1—300	Nil	1—400	1—10	1—300	1—10	Nil	1—20	Cured	Curious result with mannite. Retested it had not regained the power to ferment mannite
2790	3 days	Blood and mucus	On two plates only one purplish colony. There were numerous transparent red colonies, agglutinated by serum No 15	Acid	Acid	Acid after 3 days	Nil	Nil	+	1—200	1—50	1—200	1—100	1—1500	1—100	Nil	Nil	Died	Retested, fermented maltose and dextrin
3049	5 days	Feculent mucus	One bluish-red colony? impure. Replated, proved to be so, cultures made from non-lactose fermenters	Acid	Acid	Acid	Nil	Acid	+	1—500	1—10	1—3000	1—300	1 4000	1—500	Nil	..	Died	
3179	3 days	Bile-stained muco-pus	Some blue colonies	Acid	Acid	One strain acid after 2 days and one strain acid after 5 days	Nil	Nil	+	1—500	1—50	1—500	1—50	1—2000	1—50	Nil	1—80	Cured	Retested, fermented dextrin in 24 hours and maltose after 2 days

Number of Case	Duration of disease	Character of material received	Litmus-lactose Agar.	Dextrose	Mannite	Maltose	Saccharose	Dextrin	Indol	With serum No. 15	With serum No. 6	With Flexner serum (Lister Inst.)	With Flexner serum (P. W. & Co.)	With Hiss & Russell serum (Lister Inst.)	With Shiga serum (Lister Inst.)	With Shiga serum (P. W. & Co.)	With h. mokegeus serum	Result	Remarks
										1 in 1500	1 in 2500	1 in 1500	1 in 400 10 1 in 4000	1 in 12500		1 in 2000 1 in 4000			
3464	20 days	Mucus	Many blue colonies	Acid	Acid	Nil	Nil	Nil	+	1—200	Nil	1 400	1 200	1 1500	1—	Nil	1 100	Cured	Retested, fermented maltose after 3 days
3497	10 days	Muco-pus	Many blue colonies	Acid	Acid	Acid after 2 days	Nil	Acid after 3 days	+	1 200	1 10	1 100	1 500	1 3000	1 100	Nil	1—20	Died after operation for liver abscess	Retested, fermented maltose and dextrin in 24 hours
3500	1 month	Blood and mucus	First specimen all lactose fermenters. Second specimen some doubtful colonies	Acid	Acid	Acid after 3 days	Nil	Acid after 4 days	+	1 200	1 50	1 100	1—200	1 2000	1 —	Nil	1 50	Cured	Retested, fermented maltose and dextrin in 24 hours
3521	2 days	Mucus	From first specimen, only acid and gas organisms isolated Second specimen, a few large purplish colonies agglutinated by serum 15	Acid	Acid	Nil	Nil	Nil	+	1 300	1 50	1 400	1— 100	1 3000	1 —	Nil	1- 20	Died	Retested, maltose and dextrin not fermented
3680	13 days	Blood-streaked muco-pus	Large bright red colonies not agglutinated with serum 15. Bright blue colonies not agglutinated Small dark red colonies agglutinated by serum 15	Acid	Acid	Acid after 2 days	Nil	Nil	+	1 —200	1 50	1 1 c	1 500	1 1000	1—100	Nil	1 —	Cured	Retested, fermented maltose and dextrin in 24 hours
3682	2 days	Blood-streaked muco-pus	Mostly purplish colonies agglutinated by serum 15	Acid	Acid	Acid	Nil	Acid	+	1—200	Nil	1 500	1 300	1—1500	1 —	Nil	1 —	Absconded	
3688	10 days	Muco-pus	Practically all blue colonies not agglutinated by serum 15	Acid	Acid	Acid after 2—3 days	Nil	Two strains acid after 3 days	+	1 -50	1 10	1 50	1 -500	1—2000	1 —	Nil	1 -2 —	Cured	Retested, all strains fermented maltose and dextrin in 24 hours
3709	6 days	Muco-pus	Numerous purplish colonies agglutinated by serum 15	Acid	Acid	Acid after 3 days	One strain acid after 24 hours	Nil	+	1 -2000	1 —	1 — 500	1 1—	1 5—	—	Nil	1 —	Cured	Retested, all strains fermented maltose and dextrin in 24 hours. Saccharose was not fermented
3727	3 days	Muco-pus	Large and small reddish colonies agglutinated by serum 15	Acid	Acid	Acid after 3 days	Nil	Nil	+	1 500	1 50	1 —1500	1 400	1 1 — 1 —		Nil	1 200	Died	Retested, fermented maltose and dextrin in 24 hours

Number [illegible]	Duration of disease	Character of material received	Litmus-lactose Agar.	Dextrose	Mannite	Maltose	Saccharose	Dextrin	Indol	With serum No. 15. 1—3500	With serum No 6. 1—2000	With Flexner serum (Lister Inst.) 1—3000	With Flexner serum (B. W. & Co.) 1—2000 to 1—4000	With Hiss & Russell serum (Lister Inst.) 1—128000	With Shiga serum (Lister Inst.)	With Shiga serum (B. W. & Co.) 1—200 to 1—4000	With homologous serum.	Result.	Remarks
[illegible]	3 months	Mucus	Some purplish colonies agglutinated by serum 15	Acid	Acid	Nil	Strain (1) acid after 24 hours	Nil	+	1—300	Nil	1—700	1—400	1—6000	1—100	Nil	—	Died	Both strains gave same agglutination results. Retested strain 1 did not ferment saccharose. Maltose and dextrin not fermented
3888	5 days	Mucus	First specimen all red colonies. Second specimen two purplish colonies agglutinated by serum 15	Acid	Acid	Maltose acid after 2—3 days	One strain acid after 2 days	Acid after 2 days	+	1—2000	1—10	1—1000	1—200	1—1000	1—300	Nil	1—50	Cured	Agglutination reactions agree with all strains Retested all strains fermented Maltose after 24 hours. One strain fermented dextrin after 3 days
4110	2 days	Muco-pus	Some blue colonies not agglutinated by serum 15	Acid	Acid	Nil	Nil	Nil	+	1—200	1—10	1—300	1—500	1—500	1—100	Nil	1—20	Cured	Retested, maltose and dextrin not fermented
4304	7 days	Feculent mucus	Three specimens examined, all red colonies, agglutination nil	Acid	Acid	Acid	Nil	Acid	+	Nil	Nil	1—10	1—10	1—500	Nil	Nil	1—10	Died	
4315	15 days	Mucus	Two specimens examined. From second obtained some small reddish colonies agglutinated by serum 15	Acid	Acid	Acid after 2 days	Nil	Acid after 2 days	+	1—200	1—50	1—500	1—200	1—2000	1—100	Nil	1—20	Cured	Retested, fermented maltose and dextrin in 24 hours
4362	10 days	Feculent mucus	Numerous medium transparent reddish colonies agglutinated by serum 15	Acid	Acid	Nil	Nil	Nil	+	1—200	Nil	1—400	1—500	1—4000	1—200	Nil	1—100	Cured	Retested, maltose and dextrin not fermented
4372	15 days	Blood-streaked muco-pus	A few blue colonies agglutinated by serum 15	Acid	Acid	Acid after 3 days	Nil	Acid after 4 days	+	1—200	1—50	1—200	1—200	1—4000	1—50	Nil	Nil	Died	Retested, maltose and dextrin fermented after 24 hours
4442	5 days	Blood and muco-pus	Three specimens examined; from third obtained one or two purplish colonies agglutinated by serum 15	Acid	Acid	Acid after 2 days	Nil	Acid after 2 days	+	1—1500	1—10	1—500	1—200	1—1000	1—300	Nil	1—20	Died	Retested, maltose and dextrin fermented after 24 hours
4469	1 year	Mucus	Only one purplish colony agglutinated by serum 15	Acid	Acid	Acid after 3 days	Nil	Acid after 5 days	+	1—2000	1—10	1—500	1—300	1—500	1—300	Nil	1—100	Cured	Retested maltose fermented after 2 days and dextrin after 4 days

Summary and Conclusions.

1. Amœbæ were found in the stools of 249 out of 819 cases of dysentery admitted to District Hospital, Kuala Lumpur, during the years 1914 and 1915.

2. The stools of 63 out of 249 cases of amœbic dysentery were examined bacteriologically. 19 cases were dealt with during the first investigation and dysentery bacilli were isolated twice. 44 were dealt with during the second investigation and dysentery bacilli were not isolated.

3. The stools of 172 out of 570 cases of non-amœbic dysentery were examined bacteriologically. 105 were investigated during the period extending from the 15th May to the 15th August, 1914, and 67 were investigated during the period extending from the 1st August to the 31st December, 1915.

4. Dysentery bacilli were isolated from the stools of 72 out of the 172 cases, being 44 out of the 105 in the first series and 28 out of 67 in the second series.

5. Dysentery bacilli of the Shiga type were isolated from the stools of 8 cases.

6. Mannite-fermenting dysentery bacilli were isolated from the stools of 64 cases.

7. The mannite-fermenting dysentery bacilli were not separable into varieties or types.

8. The reactions of the mannite-fermenting dysentery bacilli on maltose, saccharose and dextrin are subject to great variations and the results should not be used either for classification or for the creation of types.

9. The dysentery bacillus of Flexner, the bacillus "Y" of Hiss and Russell and Strong's bacillus are not distinct types. These names should therefore be abolished; their retention

can only perpetuate the confusion. They should be known collectively as the mannite-fermenting dysentery bacilli.

10. The dysentery bacillus of Flexner and the bacillus "Y" of Hiss and Russell can ferment sorbite; the reaction on this substance cannot therefore be used for the purpose of classification.

11. The comparison by means of sugar-reactions of freshly isolated strains of mannite-fermenting dysentery bacilli with those which have been isolated for some time or with those isolated by other workers is unsound.

12. The reaction of the patient's serum with various strains of dysentery bacilli may be negative in cases from which dysentery bacilli have been isolated.

13. The disease associated with the mannite-fermenting dysentery bacilli does not differ from the disease associated with the *Bacillus dysenteriæ*, Shiga. The term pseudo-dysentery should therefore be abolished.

Treatment of Bacillary Dysentery.

The variety of treatments which have been recommended for this disease is evidence of their empirical character and that none of them are satisfactory.

Serum-therapy has not fulfilled expectations. Disappointing results may have been due to the use of serum in cases which were not bacillary dysentery. Other possible sources of failure are that the quantities given were inadequate or that the serum was not specific for the causative organism. The antidysenteric sera, which can be purchased, are prepared with non-mannite and mannite-fermenting strains of dysentery bacilli. The difficulties, which may be encountered in the isolation of the bacillus from many cases of dysentery, make the use of polyvalent sera unavoidable and their use consequently empirical. Any beneficial influence, which antidysenteric serum may exercise, must be temporary even when large and frequently repeated doses are administered. If the pathological condition involves large areas of the intestinal mucosa, it is useless to attempt treatment with antidysenteric serum.

A more rational procedure would be the inoculation of vaccines but these are liable to cause severe local and general disturbance. Vaccines which have been sensitized or agglutinated are free from this objection. There are two types of bacillary dysentery in this country; the less common is that caused by the Shiga's bacillus, the more common is that caused by a mannite-fermenting bacillus. By a series of experiments on animals it was found that strains of these bacilli which had been treated with specific serum were not harmful in quantities which of the untreated bacilli were harmful. Trials were made with agglutinated bacilli on cases of dysentery with equally satisfactory results.

The serum was obtained from rabbits. These animals are extremely useful for this work. A rabbit can yield a potent

serum in a month and there is no difficulty in ensuring an adequate supply. The ease and rapidity of preparation is in favour of the use of rabbits rather than of large animals such as horses. The method can be employed in any tropical laboratory and obviates the necessity of purchasing supplies of serum prepared in temperate climates from stock-cultures of dysentery bacilli. Freshness of the serum is ensured and it is made from the strains actually associated with the disease.

An agar-slope was smeared with the bacilli and incubated for 24 hours at 37°C. The culture was emulsified in 1 c.c. of sterile salt solution and the emulsion transferred to 100 c.c. of beef-broth, which was then incubated for 24 hours at 37°C. The broth-culture was sterilized by heating for one hour at 55°C and to the killed culture 0.25 c.c. of trikresol was added. 10 c.c. of serum specific for the organism was added to the killed culture and the mixture incubated for one hour at 37°C. Smaller quantities of serum would probably suffice but reduction is unnecessary. After the sterility of the product had been tested aerobically and anaerobically, it was well shaken and filled into ampoules.

The initial dose for an adult was 2 c.c. (500,000,000 killed, agglutinated bacilli), and the dose was repeated every third day. Only one case of Shiga's dysentery was treated and he left the hospital after the second injection.

Fifteen cases of dysentery caused by mannite-fermenting bacilli were treated. In only one case was tenderness noted at the site of inoculation. In no case was a febrile reaction observed and the patients were not upset. The results were perhaps better in the cases treated with their own bacillus; thus Case 3888 was cured after five injections and Case 1197 was cured after four injections. The other cases did not all do well; Case 3521 died two days after the first inoculation, but others did improve. Larger and more frequent doses will probably have to be given. Many cases of bacillary dysentery recover without treatment and

an opinion cannot be based on these few cases, but the evidence favours an extended trial of the method.

It is probable that, apart from its value as a curative agent, the treatment will confer a higher degree of immunity than that which may be derived from an unaided recovery and that the tendency to relapses or recurrences will be lessened.

In places where bacillary dysentery is prevalent, the question of prophylactic inoculations should be considered.

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